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A pharmacological review of dicoumarol: An old natural anticoagulant agent

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ABSTRACT

Dicoumarol is an oral anticoagulant agent prescribed in clinical for decades. It is a natural hydroxycoumarin discovered from the spoilage of *Melilotus officinalis* (L.) Pall and is originally discovered as a rodenticide. Due to its structural similarity to that of vitamin K, it significantly inhibits vitamin K epoxide reductase and acts as a vitamin K antagonist. Dicoumarol is mainly used as an anticoagulant to prevent thrombogenesis and to cure vascular thrombosis. Other biological activities besides anticoagulants such as anticancer, antimicrobial, antiviral, etc., have also been documented. The side effects of dicoumarol raise safety concerns for clinical application. In this review, the physicochemical property, the pharmacological activities, the side effects, and the pharmacokinetics of dicoumarol were summarized, aiming to provide a whole picture of the "old" anticoagulant.

1. Introduction

The legend of dicoumarol (3,3'-methylenebis(4-hydroxycoumarin), Fig. 1A), a coumarin-like compound, is started in 1940 when Karl Link extracted it from spoilage by fungi in sweet clover of *Melilotus officinalis* (L.) Pall (Fig. 1) [1]. *Melilotus officinalis* (L.) Pall is one of the species in the genus of Fabaceae widely distributed in Asia and Europe. In China, it has been used as a traditional Chinese herb with functions of heat-clearing and detoxifying. The medical usage of *Melilotus officinalis* (L.) Pall had also been recorded by the National Health Commission of the People's Republic of China and the European Medicines Agency in the pharmacopeias [2].

Dicoumarol also distributed in numerous plants besides *Melilotus officinalis* (L.) Pall. Its chemical structure is consisting of double benzene rings fused to lactone rings respectively [3]. The physical and chemical properties of dicoumarol are as follow:

CAS Number	66-76-2
Formula	$C_{19}H_{12}O_6$
Molar mass	336.295 g/mol
Density	1.573 g/cm^3
Melting point	290−292 °C(lit.)
Boiling point	620.702 °C at 760 mmHg
Refractive index	1.731
Solubility	Almost insoluble in water, ethanol and ether, slightly soluble in chloroform but soluble in strong alkaline solution.

Previously, dicoumarol was used as a natural anticoagulant due to its chemical structure similarity to vitamin K (Fig. 1B). Besides, dicoumarol competes with NAD(P)H for binding to NAD(P)H: quinine oxidoreductase 1 (NQO1), resulting in inhibition of NQO1 enzymatic activity. Here, we summarized the biological activities, side effects, and pharmacokinetics of dicoumarol.

2. Pharmacological activities

2.1. Anticoagulant effect

Coagulation refers to the physiological process that blood flows from a flowing liquid state to a non-flowing gel state. The essence of coagulation is the process that soluble fibrinogen in plasma changes into insoluble fibrin. Vitamin K is necessary for the liver synthesis of

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Melilotus officinalis (L.) Pall

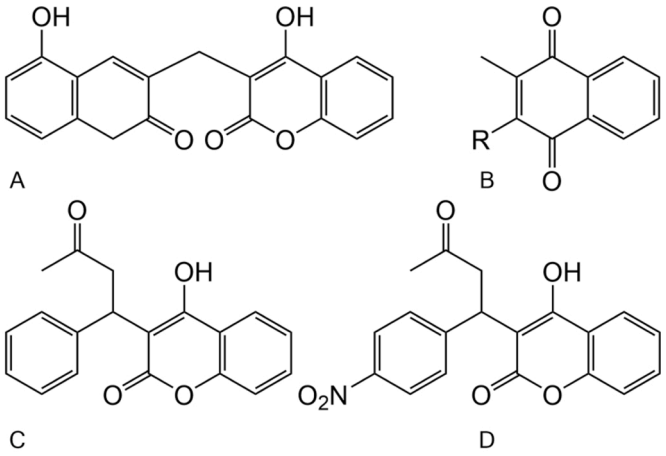

Fig. 1. The image of *Melilotus officinalis* (L.) Pall and the chemical structures of anticoagulants and vitamin K. (A) Dicoumarol; (B) Vitamin K; (C) Warfarin; (D) Acenocoumarol.

coagulation factors II, VII, IX, X, and thus antagonists of vitamin K can be used as anticoagulants in clinical practice [4].

Dicoumarol has a similar core structure with vitamin K. Hence it can competitively antagonize with vitamin K and inhibit the synthesis of coagulation factors in the liver. Further studies showed that vitamin K epoxide reductase complex subunit 1 (VKORC1), a vitamin K cyclase, is the target of dicoumarol [5]. In the meanwhile, it can stimulate red blood cells to commit suicide by stimulating Ca^{2+} entry and then triggering Ca^{2+} dependent membrane competition, leading to anemia [6].

The discovery of dicoumarol is due to its outstanding anticoagulant effect which is also the main traditional pharmacological effect [7]. Because dicoumarol inhibits the synthesis of coagulation factors, its anticoagulant effect is slow in the beginning due to there are enough coagulation factors in the blood. However, it can last for a long time when these coagulation factors were depleted. In the clinic, dicoumarol is used to prevent the formation and development of thrombus. When acute myocardial obstruction occurs, surgery or thrombolytics are generally used to improve blood flow. To prevent a recurrence, patients generally need to take antiplatelet and anticoagulant drugs for long-term. As an anti-coagulant, dicoumarol has been used clinically to prevent the recurrence of myocardial infarction [8,9]. However, it was quickly replaced by its derivative warfarin, which shows more stable pharmacokinetic parameters [10]. Now, in terms of myocardial infarction, dicoumarol is only used as an alternative drug for patients with warfarin intolerance. Since the discovery of dicoumarol, a series of vitamin K antagonists with the parent structure of "coumarin" have been identified and synthesized. Warfarin (Fig. 1C) is the representative one, which passed clinical trials in the 1950s. Compared with dicoumarol, warfarin is more potent and has superior pharmacokinetic parameters [11]. Currently, most clinical anticoagulant agents are coumarins, such as warfarin, dicoumarol, and acenocoumarol (Fig. 1D) [12,13].

2.2. Anticancer effect

Dicoumarol exerts an inhibitory effect on cell viability, cell proliferation, and induces apoptosis in a variety of cancers such as cholangiocarcinoma, osteosarcoma, mastadenoma, leucocythemia, renal carcinoma, and melanoma. Dicoumarol ligand with sodium adduct exerts cytotoxicity in U2OS human bone osteosarcoma epithelial cells [14]. Dicoumarol arrests the cell cycle in G0/1 phase by increasing the cellular superoxide in HL-60 human myeloid leukemia cells [15]. It induces apoptosis of cancer cells in a concentration- and time-dependent manner, which was mediated by oxidative stress, cytochrome c release followed by activation of caspase-9 and cleavage of caspase-3 [16–18]. Furthermore, it induces apoptosis in MCF-7 breast cancer cells but shows no toxicity on oocyte maturation and ovarian tissues of the mouse [19].

Furthermore, dicoumarol considerably enhanced the cytotoxicity of ascorbate and doxorubicin in MCF-7 cells by inhibiting NQO1 [20] and it also enhanced the cytotoxicity of doxorubicin in urothelial cancer cells through p53/p21/p38 MAPK pathway [21], respectively. The enhancement may due to its inhibitory effect on NQO1. In estrogen receptor-negative breast cancer, dicoumarol can inhibit chemoresistance to TA-based chemotherapy by targeting the Pregnancy specific beta-1-glycoprotein 1 (PSG1) [22]. Dicoumarol enhanced the cytotoxicity of gemcitabine in human cholangiocarcinoma cells with high NQO1 activity but not in low NQO1 activity cells [23]. Dicoumarol promotes miltirone-induced intracellular ROS and destabilizes p53 stability through NQO1 inhibition in HCT116 human colon cancer cells [24]. Dicoumarol suppresses cell migration and enhanced the inhibitory effect of 5-FU in high mobility group protein AT-hook2 overexpressing DLD-1 human colon cancer cells [25].

However, dicoumarol can compromise the anticancer effect of those drugs targeting NQO1 [26]. Recently, we proposed a novel type of programmed necrosis termed as noptosis which is mediated by NQO1 and NQO1 activation-derived reactive oxygen species (ROS). Naphthoquinones are potent inducers of noptosis [27]. Dicoumarol is an effective and specific inhibitor of noptosis by inhibiting NQO1. Dicoumarol reduced the cytotoxicity of tirapazamine in NQO1 expression A549 cells [28]. It nearly completely reverses 2-methoxy-6-acetyl-7-methyljuglone, a natural naphthoquinone, induced non-apoptotic necrosis in U87 and U251 glioblastoma cells [29]. The cytotoxicity effects of EO9, a prodrug that needs metabolical activation by NQO1, in HT29, A549, and MIA PaCa-2 cancer cells were decreased by dicoumarol [30]. Dicoumarol also restrains the cytotoxicity of the β-lapachone via inhibited NQO1 activity [31].

2.3. Antimicrobial and antiviral activities

Dicoumarol and a series of its derivatives containing Cu^{2+} complexes exerted the antibacterial activity including gram-positive bacterium (*Bacillus subtilis* ATCC11774 and *Streptococcus pyogenes* ATCC12384), gram-negative bacterium (*Pseudomonas aeruginosa* ATCC25619 and *Escherichia coli* ATCC25922) and *Mycobacterium tuberculosis* H37Ra. It also exerts antifungal activity like *Aspergillus niger* ATCC64958 and *Candida albicans* ATCC66027 [32,33]. 2-Pyridinodicoumarol (2-PyDC), a dicoumarol derivative, exerts antibacterial activity in four *Staphylococcus aureus* bacterial strains, including *S. aureus* ATCC 29213, MRSA XJ 75302, Mu50 and USA 300 LAC with the minimal inhibitory concentration (MIC) ranging from 16 to 64 μg/mL [34]. Dicoumarol inhibits *E. coli* nitroreductase enzyme, an activating enzyme for nitroaromatic prodrugs of the dinitrobenzamide class [35]. In addition, the structure-activity relationships (SAR) of dicoumarol derivatives have

revealed that the types and the position of substituents on the benzene ring have a strong effect on anti-*staphylococcus aureus* activities [36].

Dicoumarol was reported to exert the anti-human immunodeficiency viruses (HIV) ability. Tat, one of the HIV-1 encoded regulatory proteins, exerts the steady-state levels maintains ability in viral transcripts. Dicoumarol inhibits HIV-1 replication by degrading Tat level through inhibiting NQO1 [37]. Dicoumarol derivatives also exerted extraordinary anti-HIV activity [38].

2.4. Inhibition of NQO1

NQO1 is a type of flavinase enzyme that regards NAD(P)H as a receptor to catalase the reduction reaction of quinone compounds by losing two electrons. It plays an irreplaceable function in the process of material and energy metabolism in cells including detoxification of xenobiotic, superoxide elimination, p53 modulation, and proteasomal degradation, of which could be inhibited by dicoumarol [39].

NQO1 is a homodimer containing double active sites which located in the surface of subunits, FAD acts as the cofactor role of one active site, the substrate parallels to FAD and combines with NQO1 to be catalyzed. Dicoumarol exerts the NQO1 inhibitor function by partly overlapping FAD thus inhibiting the electron transfer from the enzyme to the substrate [40].

2.5. Other activities

Aristolochic acids are a group of carcinogenic, mutagenic, and nephrotoxic compounds widely distributed in the plant kingdom, including many traditional Chinese medicines. Dicoumarol pretreatment attenuates aristolochic acid I (AAI) induced nephrotoxicity by blocking NQO1 activity to participate in the nitroreduction of AAI in C57BL/6 mice [41]. However, dicoumarol may have a dual role in NQO1-mediated genotoxicity of AAI and was found to be an inducer of NQO1 in the kidney and lung of rats. It suppresses the formulation of AAI-DNA adduct by acting as an NQO1 inhibitor but increases the activation level of AAI-DNA adduct by acting as an NQO1 inducer [42]. Thus, its precise role in the nephrotoxic and carcinogenic of AAI remains to be clarified.

Dicoumarol strongly affects the GSH metabolism by inhibiting the cellular multidrug resistance protein (Mrp) 1-dependent GSH export in cultured astrocytes with a different mechanism to that of the known MRP1 inhibitor MK571 [26]. Dicoumarol inhibits steroid biotransformation stage II and significantly inhibits progesterone inactivation in cultured pigs and cows hepatocytes mediated by inhibition of uridine diphosphate-glucuronosyltransferase [43]. As an inhibitor of ADP-ribosylation of CtBP3/BARS, dicoumarol antagonizes brefeldin-A-dependent Golgi tubulation and redistribution to the endoplasmic reticulum and selectively breakdown Golgi non-compact tubular zones and inhibits the transport in Golgi apparatus [44]. Dicoumarol effectively inhibits connexin-43-dependent gap junctional intercellular communication in rat liver epithelial cells or human skin fibroblasts paralleled by a reversible loss of a phosphorylated form of connexin-43 [45]. Menadione (2-methyl-1,4-naphthoquinone) shows selectively toxicity in erythrocytes causing haemolytic anaemia *in vivo*. Dicoumarol increased the severity of haemolytic anaemia by inhibiting NQO1 in rats [46].

It is deserved to note that the cytotoxic effect of dicoumarol detected by MTT assay and clonogenic assay are opposite [47] and there is also a difference between MTT assay and Alamar Blue assay [48]. Mechanisms for this inconsistency remain unclear and might due to the production of ROS and the uncoupling of mitochondria [47] and the influence of some metabolic enzymes responsible for the transformation of cytotoxic endpoints [48]. Thus, the cytotoxic effect of dicoumarol obtained from MTT assay might need to be confirmed by other methods to avoid misleading results.

3. Side effects

The side effects of dicoumarol and other vitamin K antagonists have been well documented due to a long time of clinical application.

3.1. Hemorrhagic complications

The anticoagulant effect of dicoumarol causes the side effects of internal hemorrhage and anemia during use, and this is also the main mechanism of dicoumarol as rodenticide [49]. Hemorrhage is the most common side effect of dicoumarol and can lead to damage to many tissues and organs, which shows different clinical manifestations. The international normalized ratio (INR) is the internationally recognized expression mode for monitoring the dosage of oral anticoagulants [50]. Clinically, the target INR is 2.0–3.0 when using warfarin, dicoumarol, or other drugs for oral anticoagulant treatment, which can not only ensure the treatment effect but also maintain the risk of hemorrhage at a lower level [51]. In a clinical study of 75 years or older patients with non-valvular atrial fibrillation, a total of 193 patients received dicoumarol. The incidence of any bleeding was 13.7/100 person-years and corresponding figures for major bleeding were 3.3/100 person-years for those on dicoumarol [52].

Skin necrosis caused by skin hemorrhage is one of the serious complications reported for many times [53]. The incidence of coumarin induced skin necrosis ranged from 0.01 % to 0.1 % [54]. It often occurs in female patients, typically develops in regions with abundant subcutaneous fat, such as breast, abdomen, buttock, and thigh [55]. There are also cases of adrenal hemorrhage [56,57] and ovarian hemorrhage [58]. In the digestive system, the hemorrhage in the intramural intestinal tracts will lead to hematoma and Ileus in the small intestine [59,60]. In the respiratory system, there are clinical reports revealed that patients have airway obstruction after taking dicoumarol due to spontaneous retropharyngeal hemorrhage [61].

Therefore, no matter what kind of disease dicoumarol will be used for in the future, it is necessary to take precautions in advance for the hemorrhagic complications, and the elderly patients with complications or patients taking combination drugs may need close clinical examination.

3.2. Neurotoxicity

The neurotoxicity of dicoumarol may result from its inhibition of NQO1, which plays a protective role in the dopaminergic system of the substantia nigra striatum [62]. Thus, as an inhibitor of NQO1, dicoumarol may increase the damage to the nigrostriatal dopaminergic system when used in combination with other medicines. For instance, a combination of aminochrome and dicoumarol for 48 h induced approximately 70 % cell death in the RCSN-3 neuronal dopaminergic cell line [63]. Copper, a cofactor of several enzymes, has obvious neurotoxicity related to the oxidation of dopamine to aminochrome. Dopamine-dependent copper neurotoxicity is enhanced by dicoumarol, which is mediated by NQO1 inhibition in RCSN-3 cells [64]. Dicoumarol increased the neurotoxic side effects of salbutamol, a commonly used anti-asthma drug, in RCSN-3 cells as well [65], which should arouse the attention in clinical medication. However, dicoumarol alone shows no significant toxic effect on primary astrocytes and RCHT rat hypothalamus cells [26,65]. Thus, the prescription of dicoumarol and its analogs should be carefully considered for the treatment of coagulation symptoms in Alzheimer's patients in combination treatment.

3.3. Periodontal toxicity

Dicoumarol increased the toxicity of areca nut extract to gingival keratinocytes by inhibiting the ANE-induced PGE2 production [66]. Given the accumulated evidence of areca nut in the etiopathogenesis of oral cancer and submucous fibrosis [67,68], chronic dicoumarol

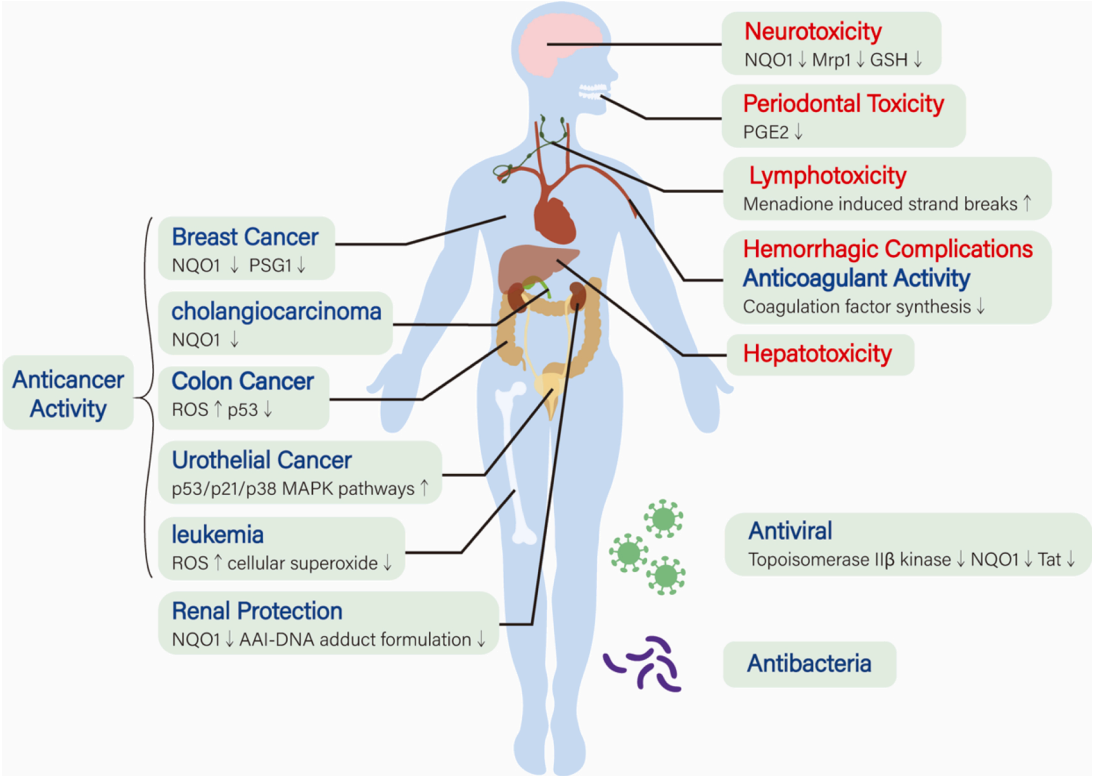

Fig. 2. The pharmacological effect, side reactions, and potential mechanisms of dicoumarol. As an anticoagulant from the natural source, dicoumarol demonstrates various biological activities. However, some side reactions have already been documented after decades of clinical application.

treatment may increase the risk of oral cancer and oral submucous fibrosis in areca-nut chewing patients. A survey showed that patients who take dicoumarol orally for anticoagulant treatment have different degrees of periodontal disease, especially gingivitis related to bacterial plaque [69]. Patients with anticoagulant therapy are more likely opposed to take toothbrushing owing to bleeding frequently, which rising the risk of oral infection with bacteria and leading the formation of bacterial plaque [70]. Thus, persistent long-term treatment with dicoumarol may increase the risk of periodontal disease [71].

3.4. Hepatotoxicity and lymphotoxicity

Liver-specific side effects are rare in dicoumarol treatment, but there are reports of inflammatory infiltration, hepatocyte necrosis, and liver failure. Therefore, transaminase and cholestasis parameters should be checked regularly. If there is unclear liver disease, the medication treatment should be weaned off immediately [54,72]. Dicoumarol enhanced the production of menadione induced strand breaks in isolated human lymphocytes [73]. In the absence of serum, dicoumarol showed significant cytotoxicity to HL-60 cells [74].

4. Pharmacokinetics

4.1. Animal data

The pharmacokinetics of dicoumarol in rats show dramatic interindividual differences, which might due to the pronounced differences in the serum protein binding of dicoumarol [75]. Different from warfarin, after received single injections the free fraction of dicoumarol in rats serum is less than that in the rat liver [76]. The biotransformation of dicoumarol mainly occurs in the liver [77], and the biological half-life and apparent volume of distribution of dicoumarol in rats are smaller than that of warfarin. The biological half-life of dicoumarol ranged from 5 h to 28 h [75]. The total clearance of dicoumarol was determined in 172 adult male Sprague-Dawley rats. Clearance values ranged from 1.46 to 27.0 mL/h/kg. The frequency distribution curve for dicoumarol total clearance is very similar to the trimodal frequency distribution curve for warfarin serum-free fraction values in rats [78].

4.2. Clinical research

The mean half-life of dicoumarol in the plasma of monozygotic and monozygotic twins after a single oral dose of 4 mg/kg was 43.6 h. Significant differences between subjects were controlled by heredity rather than the environment. This means that the monitoring of drug metabolism might be necessary for the clinic. After gradually increasing the dose, it was found that the half-life of dicoumarol concurrently increased. The dose dependence of the drug half-life led to an increase in the variability of the half-life, so it had a greater risk of toxicity for long-term treatment [79].

The bioavailability of dicoumarol is very poor partially due to its low solubility in water. Improve bioavailability in animal models or humans has been achieved by increasing its solubility through different methods, such as the preparation of solid dispersion system, the preparation of nanospheres, themicrospheres by adding bioadhesive polymer and the freeze-dried formulations [80–82]. However, due to the wide application of warfarin in clinical practice, none of these pharmaceutical dosage forms of dicoumarol is available on the market.

Dicoumarol can affect the fetus through the blood placenta barrier and a high dose can lead to hemiplegia and brain material loss. Dicoumarol is relatively taboo during pregnancy while warfarin sodium, which will not be excreted into breast milk, can be used in the postpartum anticoagulant therapy for lactating women. However, dicoumarol is the first choice for patients who are intolerant to warfarin sodium [83].

The main pharmacological and toxic effects and potential mechanisms are summarized in Fig. 2.

5. Conclusion

Serendipity plays a role in the discovery of several drugs [84]. The discovery of dicoumarol as an anticoagulant is an example, which opens the development of coumarins anticoagulant drugs, such as warfarin, acenocoumarol, ethyl biscoumacetate, etc. The main mechanism of this category of drug is by interfering with the metabolism of vitamin K. Beside its anticoagulant effect, dicoumarol and its derivatives show anticancer, antimicrobial, antiviral activities. Furthermore, other targets for dicoumarol such as NQO1 have been identified, which might provide potential applications other than an anticoagulant. However, the side reactions, especially hemorrhagic complications, need to be avoided in clinical practice though it has been used for several decades.

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Declaration of Competing Interest

The authors report no declarations of interest.

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